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# African Great Apes Are Naturally Infected with Roseoloviruses Closely Related to Human Herpesvirus 7

**Anne Lavergne,[a] Damien Donato,[a] Antoine Gessain,[b] Henk Niphuis,[c] Eric Nerrienet,[d] Ernst J. Verschoor,[c] Vincent Lacoste[a]**

Laboratoire des Interactions Virus-Hôtes, Institut Pasteur de la Guyane, Cayenne, French Guiana[a]; Unité d'Epidémiologie et Physiopathologie des Virus Oncogènes, Institut Pasteur, Paris, France[b]; Department of Virology, Biomedical Primate Research Centre (BPRC), Rijswijk, The Netherlands[c]; Centre Pasteur du Cameroun, Yaoundé, Cameroon[d]

**ABSTRACT**

**Primates are naturally infected with herpesviruses. During the last 15 years, the search for homologues of human herpesviruses in nonhuman primates allowed the identification of numerous viruses belonging to the different herpesvirus subfamilies and genera. No simian homologue of human herpesvirus 7 (HHV7) has been reported to date. To investigate the putative existence of HHV7-like viruses in African great apes, we applied the consensus-degenerate hybrid oligonucleotide primers (CODEHOP) program-mediated PCR strategy to blood DNA samples from the four common chimpanzee subspecies (*Pan troglodytes verus*, *P. t. ellioti*, *P. t. troglodytes*, and *P. t. schweinfurthii*), pygmy chimpanzees (*Pan paniscus*), as well as lowland gorillas (*Gorilla gorilla gorilla*). This study led to the discovery of a novel roseolovirus close to HHV7 in each of these nonhuman primate species and subspecies. Generation of the partial glycoprotein B (1,111-bp) and full-length DNA polymerase (3,036/3,042-bp) gene sequences allowed the deciphering of their evolutionary relationships. Phylogenetic analyses revealed that HHV7 and its African great ape homologues formed well-supported monophyletic lineages whose topological resemblance to the host phylogeny is suggestive of virus-host codivergence. Notably, the evolutionary branching points that separate HHV7 from African great ape herpesvirus 7 are remarkably congruent with the dates of divergence of their hosts. Our study shows that African great apes are hosts of human herpesvirus homologues, including HHV7 homologues, and that the latter, like other DNA viruses that establish persistent infections, have cospeciated with their hosts.**

**IMPORTANCE**

**Human herpesviruses are known to possess simian homologues. However, surprisingly, none has been identified to date for human herpesvirus 7 (HHV7). This study is the first to describe simian homologues of HHV7. The extensive search performed on almost all African great ape species and subspecies, i.e., common chimpanzees of the four subspecies, bonobos, and lowland gorillas, has allowed characterization of a specific virus in each. Genetic characterization of the partial glycoprotein B and full-length DNA polymerase gene sequences, followed by their phylogenetic analysis and estimation of divergence times, has shed light on the evolutionary relationships of these viruses. In this respect, we conclusively demonstrate the cospeciation between these new viruses and their hosts and report cases of cross-species transmission between two common chimpanzee subspecies in both directions.**

The *Betaherpesvirinae* subfamily belongs to the *Herpesviridae* family, order *Herpesvirales* (1). This subfamily consists of four genera: *Cytomegalovirus* (CMV), *Muromegalovirus*, *Roseolovirus*, and *Proboscivirus*. While the *Cytomegalovirus* and *Roseolovirus* genera are found in primates, including humans, *Muromegalovirus* and *Proboscivirus* have rodents and elephants as hosts, respectively. During the last few decades, Old World primates, including great apes, and New World primates have been studied extensively and found to harbor several herpesviruses (2–4). This led to the identification of numerous lymphocryptoviruses and rhadinoviruses of the *Gammaherpesvirinae* subfamily, as well as cytomegaloviruses from different nonhuman primate (NHP) species (5–17).

Among the primate herpesviruses, roseoloviruses are the closest relatives of CMVs. The prototypic *Roseolovirus* is human herpesvirus 6 (HHV6). This virus was first isolated in 1986 from the peripheral blood of patients with lymphoproliferative disorders (18, 19). Soon after its discovery, two distinct variants, HHV6A and HHV6B, were recognized and are now classified as separate viruses (20). In addition, shortly following the discovery of HHV6, antibodies reacting with HHV6 antigens were detected in sera from eight different monkey species (21). The first molecular proof of the existence of a roseolovirus-like virus in NHP was the characterization of a short DNA sequence of the herpesviral DNA polymerase gene from mandrill and drill monkeys (5). This virus, named mandrill herpesvirus β (MndHVβ), was only distantly related to the human roseoloviruses. In 2005, we described the first HHV6-related virus in common chimpanzees (22). No other simian homologue of HHV6 has been characterized since then. The other human roseolovirus, human herpesvirus 7 (HHV7), was identified in 1990 from the stimulated $CD4^+$ T cells of a healthy

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Address correspondence to Vincent Lacoste, vlacoste@pasteur-cayenne.fr.
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**TABLE 1** African great apes tested for HV7 by molecular methods and survey results

| Subspecies or species | Origin | Status | No. of animals | No. of positive animals[a] | % of positive animals | Name proposal | |
|---|---|---|---|---|---|---|---|
| | | | | | | Virus name | Acronym |
| *Pan troglodytes verus* | The Netherlands | Colony animals | 26 | 9 | 35 | *Pan troglodytes* herpesvirus 7*v.* | PtroHV7*v.* |
| *Pan troglodytes ellioti* | Cameroon | Wild-caught animals | 10 | 4 | 40 | *Pan troglodytes* herpesvirus 7*e.* | PtroHV7*e.* |
| *Pan troglodytes troglodytes* | Cameroon | Wild-caught animals | 10 | 2 | 20 | *Pan troglodytes* herpesvirus 7*t.* | PtroHV7*t.* |
| *Pan troglodytes schweinfurthii* | The Netherlands | Colony animals | 17 | 6 | 35 | *Pan troglodytes* herpesvirus 7*s.* | PtroHV7*s.* |
| *Pan paniscus* | The Netherlands | Zoo animals | 21 | 13 | 62 | *Pan paniscus* herpesvirus 7 | PpanHV7 |
| *Gorilla gorilla gorilla* | The Netherlands | Zoo animals | 14 | 11 | 78 | *Gorilla gorilla* herpesvirus 7 | GgorHV7 |

[a] As determined by PCR amplification with specific primers (ASA/ASB1 and ASA/ASB2).

individual (23). This virus is transmitted early in life via saliva, inducing in most cases an asymptomatic primary infection (24, 25). However, in some cases, it can induce an exanthem subitum or even, more rarely, severe symptoms. In all cases, this results in a lifelong chronic infection. HHV7 is a ubiquitous virus with a seroprevalence exceeding 90% in the adult population (26, 27). HHV7 was considered a benign virus, but the spectrum of human diseases possibly related to it has widened (28, 29). Its capacity to induce severe illnesses is now recognized, especially in transplant recipients (30, 31).

Paradoxically, HHV7 is the only human virus for which no simian homologue has been identified. In our opinion, there was little doubt that HHV7 also possessed simian homologues. To address the question of the natural infection of NHP with HHV7-related viruses, we screened blood DNA samples from common chimpanzees of the four subspecies, bonobos, and gorillas. By applying the consensus-degenerate hybrid oligonucleotide primers (CODEHOP) program-mediated PCR strategy (32), we were able to identify HHV7-like viruses from each of these great ape species and subspecies. Partial sequences of the glycoprotein B gene and full-length sequences of the DNA polymerase gene of these HHV7 homologues have been generated from each host species. These data enabled us to gain insights into their occurrence, genetic diversity, and evolutionary history.

## MATERIALS AND METHODS

**Animals.** In previous studies on retroviruses and herpesviruses of NHPs, we obtained blood DNA samples from common and pygmy chimpanzees, as well as gorillas (6, 7, 33–35). We tested samples from a total of 63 common chimpanzees (26 *Pan troglodytes verus*, 10 *P. t. ellioti*, 10 *P. t. troglodytes*, and 17 *P. t. schweinfurthii* chimpanzees), 21 pygmy chimpanzees (*Pan paniscus*), and 14 Western lowland gorillas (*Gorilla gorilla gorilla*) (Table 1). While the *P. t. verus* and *P. t. schweinfurthii* samples were obtained from individuals living in closed breeding colonies housed at the Biomedical Primate Research Centre (BPRC), Rijswijk, The Netherlands, those from *P. paniscus* and *G. g. gorilla* were archived blood samples from zoo animals sent to BPRC for viral diagnostic testing (35). Samples from the *P. t. troglodytes* and *P. t. ellioti* subspecies were collected from wild-caught individuals, most of which were housed in a rehabilitation center in Cameroon (6, 33, 34). For each animal, high-molecular-weight DNA was obtained either from peripheral blood mononuclear cells or from buffy coat using a QIAamp DNA blood minikit (Qiagen GmbH, Hilden, Germany). This study was based on samples collected several years ago, and so no approval was required.

**Initial screening of samples.** Molecular screening was done by seminested PCR (snPCR) amplification with degenerate consensus primers targeting highly conserved amino acid motifs of the herpesvirus DNA polymerase gene, known as open reading frame (ORF) *U38* for HHV7 (see Table S1 in the supplemental material). To maximize our chances of amplifying HHV7-like sequences, the primers of Rose et al. were refined from alignments of primate cytomegalovirus and roseolovirus DNA polymerase sequences available in the databases (10). In addition, to avoid amplification of cytomegaloviruses, the primers were only minimally degenerated. The DFASA7, VYGA7, and GDTDB7 primers were designed and used in place of the DFASA, VYGA, and GDTD1B primers, respectively. The DFASA7/GDTDB7 primers were used on each DNA sample for the first-round PCR. Then, the VYGA7/GDTDB7 primers were used in the second-round PCR. PCR analyses were performed using the thermostable DNA polymerase AmpliTaq Gold (error rate, $2.6 \times 10^{-5}$; Life Technologies, Carlsbad, CA, USA) at an annealing temperature of 60°C with an elongation time of 30 s for 35 cycles. When detected, amplicons of the predicted size were purified, cloned by TA cloning, and sent for sequencing to Beckman Coulter Genomics, Takeley, United Kingdom.

**Herpesvirus glycoprotein B and DNA polymerase gene amplification.** To obtain the nucleotide sequence upstream of the VYGA7 motif, a set of species-specific nondegenerate primers (PtB, PpB, and GgB) was derived from the complementary sequences of the small fragments and used in a nested PCR amplification with the DFASA7 primer pool using the initial PCR products (DFASA7 and GDTDB7) as the templates (see Table S1 in the supplemental material). Then, the partial glycoprotein B (ORF *U39*) and full-length DNA polymerase gene sequences were obtained using different sets of consensus degenerate and species-specific primers designed using a primate betaherpesvirus glycoprotein B, DNA polymerase, and *U37* gene sequence alignment (see Table S1 in the supplemental material). Overlapping amplicons were generated, cloned, and sequenced. Each sequence corresponds to at least three independent clones sequenced on both strands. Contig sequences of the glycoprotein B and DNA polymerase genes were then assembled using MEGA (v5.05) software (36).

**Specific screening for prevalence.** To determine the prevalence of the different HHV7 homologues in their respective host species, a set of three nondegenerate consensus primers (ASA, ASB1, and ASB2) was designed by identifying conserved regions between the different African great ape viruses from alignments of the DNA polymerase gene sequences obtained (see Table S1 in the supplemental material). They allowed screening of all African great apes via a seminested PCR approach (PCR with primers ASA and ASB1 followed by PCR with primers ASA and ASB2), whose detection limit has been determined to be at the nanogram level of starting material.

**Phylogenetic analysis.** Raw sequences were analyzed and edited in MEGA (v5.05) (36). Multiple-sequence alignments with other previously published primate *Roseolovirus* sequences were constructed using the ClustalW program, and the alignments were checked manually. The sequences were translated into amino acids, and both nucleotide and amino acid sequences were checked for irregularities. Hypervariable regions generating a suboptimal alignment were removed before performing phylogenetic analyses. Phylogenetic trees were inferred from the aligned amino acid sequences. The ProtTest3 program was used to determine the optimal model of amino acid evolution, and the JTT model with a gamma (G) distribution was identified and used for the Bayesian approach (37), which was performed with the program Mr. Bayes (v3.2.2) to infer phylogenetic relationships (38). Markov chain Monte Carlo (MCMC) simu-

**TABLE 2** Nucleotide and amino acid identities between the different great ape roseoloviruses on the basis of glycoprotein B partial gene sequence

| Virus | GenBank accession no. | % identity with[a]: PtroHV7*v.* Nucleotide | PtroHV7*v.* Amino acid | PtroHV7*s.* Nucleotide | PtroHV7*s.* Amino acid | PpanHV7 Nucleotide | PpanHV7 Amino acid | GgorHV7 Nucleotide | GgorHV7 Amino acid |
|---|---|---|---|---|---|---|---|---|---|
| PtroHV7*v.* | KJ843227 | | | 98.3 | 97.8 | 94.1 | 96.7 | 83.8 | 85.1 |
| PtroHV7*s.* | KJ843228 | 98.3 | 97.8 | | | 94.4 | 96.7 | 83.6 | 85.4 |
| PpanHV7 | KJ843229 to KJ843230 | 94.1 | 96.7 | 94.4 | 96.7 | 100.0 | 100.0 | 83.8 | 86.7 |
| GgorHV7 | KJ843231 to KJ843232 | 83.8 | 85.1 | 83.6 | 85.4 | 83.8 | 86.7 | 100.0 | 100.0 |
| HHV7 strain RK | AF037218 | 85.1 | 89.4 | 84.8 | 88.9 | 85.1 | 89.2 | 84.1 | 88.1 |
| HHV7 strain JI | U43400 | 85.1 | 89.4 | 84.7 | 88.9 | 85.1 | 89.2 | 84.0 | 88.1 |
| HHV6A strain U1102 | X83413 | 59.1 | 56.0 | 59.0 | 56.5 | 59.1 | 56.9 | 59.2 | 55.6 |
| HHV6B strain Z29 | AF157706 | 58.9 | 56.0 | 59.1 | 56.5 | 59.4 | 56.9 | 59.0 | 56.1 |
| *Pan troglodytes* roseolovirus 1 | AY854171 | 60.3 | 56.8 | 60.4 | 57.3 | 60.7 | 57.8 | 59.8 | 56.6 |

[a] The viral strains were compared on the basis of the common 1,111-bp/369-aa fragments.

lations were run for 10,000,000 generations with four simultaneous chains, using a sample frequency of 100 and a burn-in of 25,000. Majority rule consensus trees were obtained from the output. Validation of the inference was assessed on the basis of the standard deviation of split frequencies, which was less than the expected threshold value of 0.01 (calculated value, 0.002).

**Time calibration.** Divergence times between clades were calculated using a relaxed Bayesian molecular clock model with an uncorrelated lognormal rate of distribution, as implemented in the BEAST (v1.7.4) program (39). A monophyletic constraint was imposed for the node used to calibrate evolutionary rates. The time of the most recent common ancestor (tMRCA) of the human-chimpanzee and gorilla clades, estimated to be between 7 and 12 million years ago (MYA; 9.5 MYA; standard deviation, 1.5 MYA), was used as the single calibration point. This date is based on molecular calibration (40). The amino acid substitution model was the same as the one described above. We ran analyses assuming that the population size has grown exponentially. Results were obtained for 10,000,000 generations, with the results for the first 2,000,000 generations being discarded as burn-in, and parameter values were sampled every 100 generations. The effective sample size for parameter estimates and convergence were checked using Tracer (v1.5.0) software (41). The final tree with divergence estimates and their 95% highest posterior densities (HPDs) was computed in the TreeAnnotator (v1.4.5) program (39).

**Nucleotide sequence accession numbers.** The partial glycoprotein B and complete DNA polymerase gene sequences reported in this paper have been deposited in the GenBank database under accession numbers KJ843227 to KJ843244.

## RESULTS

HHV7-like viruses in African great apes were sought by attempting to amplify a 237-bp fragment of the highly conserved herpesvirus DNA polymerase gene from peripheral blood mononuclear cell DNA of each NHP of our collection using the PCR conditions previously described (5, 6). Samples from a total of 98 apes belonging to the four *P. troglodytes* subspecies and the *P. paniscus* and *G. g. gorilla* species were tested (Table 1). Several DNA samples from each species and subspecies scored positive on the ethidium bromide gels after the snPCRs (Table 1; see also Table S2 in the supplemental material). Sequencing of three individual clones from at least two randomly chosen individuals of each species or subspecies allowed us to identify herpesvirus sequences. All sequences were novel, belonged to the *Roseolovirus* genus, and were most similar to that of HHV7, as observed in BLAST database searches (http://www.ncbi.nlm.nih.gov/BLAST/).

We then used different couples of consensus degenerate and specific PCR primers to generate a contig sequence of the partial glycoprotein B (1,111-bp) and full-length DNA polymerase (3,036/3,042-bp) genes from at least one representative of each ape species (one *P. t. verus*, one *P. t. schweinfurthii*, two *P. paniscus*, and two *G. g. gorilla* individuals) (see Tables S1 and S2 in the supplemental material). Furthermore, one to three additional contig sequences of the complete DNA polymerase gene only were generated for each species and subspecies. In total, 17 complete DNA polymerase gene sequences plus 1 partial sequence from a *P. t. troglodytes* individual were obtained (see Table S2 in the supplemental material). BLAST searches demonstrated that all sequences identified were more closely related to HHV7 than to any other known herpesvirus and, in addition, revealed the presence of host species-specific sequences. We propose to name the common chimpanzee virus PtroHV7, for *Pan troglodytes* herpesvirus 7 (HV7); we propose to name the pygmy chimpanzee virus PpanHV7, for *Pan paniscus* HV7; and we propose to name the Western lowland gorilla virus GgorHV7, for *Gorilla gorilla gorilla* HV7. Informal virus names and abbreviations are reported in Table 1.

To assess the prevalence of these new viruses, we set up a semi-nested PCR assay using nondegenerate primers to generically detect HV7 DNA polymerase sequences in our collection of African great ape DNA samples (see Table S1 in the supplemental material). Among the 84 chimpanzees, 34 scored positive on the ethidium bromide-stained gel: 13 for *P. paniscus*, 9 for *P. t. verus*, 4 for *P. t. ellioti*, 2 for *P. t. troglodytes*, and 6 for *P. t. schweinfurthii*. For gorillas, 11 out of the 14 animals gave a PCR product of the expected size. These results perfectly correlated with the data obtained using our initial PCR screening approach with degenerate primers (see Table S2 in the supplemental material). This represents an overall prevalence of 45.9%, with the lowest (20%) being for *P. t. troglodytes* and the highest (78%) being for *G. g. gorilla* (Table 1). At the host species level, the lowest prevalence (33.3%) was obtained for *P. troglodytes*, whereas the prevalence was 62% for *P. paniscus* and 78% for *G. g. gorilla*.

The partial glycoprotein B sequences were 1,111 bp in length, while the full-length DNA polymerase sequences were 3,036 bp (*G. g. gorilla*) and 3,042 bp (*P. troglodytes* and *P. paniscus*) long. Glycoprotein B sequences exhibited 84.0 to 85.1% nucleotide sequence identities and 88.1 to 89.4% amino acid (aa) sequence identities with the JI and RK strains of HHV7 (Table 2). The level of nucleotide and amino acid sequence identity was below 60%

with the U1102 and Z29 strains of HHV6. Furthermore, pairwise nucleotide sequence comparisons of the full-length DNA polymerase genes confirmed that they were all more closely related to the corresponding sequences of HHV7 (from 85.7% for GgorHV7 to 86.5% for PtroHV7) than to the corresponding sequences of HHV6 (range, 64.4% for the sequence of PpanHV7 versus that of HHV6A strain U1102 to 65.1% for the sequence of GgorHV7 versus the sequences of HHV6B strains) (Table 3). Similar results were obtained when the comparison was made on the basis of the deduced amino acid sequences. In addition, the viral DNA polymerase gene sequences obtained from the four *P. troglodytes* subspecies were 98.3 to 99.6% identical to each other, 94.8 to 95.6% identical to the gene sequence from *P. paniscus*, and 86.7 to 87.4% identical to the gene sequence from *G. g. gorilla* (Table 3). At the protein level, the DNA polymerase sequences from the four *P. troglodytes* subspecies also showed 98.3 to 99.6% identity to each other. Despite the high level of protein sequence conservation between the different PtroHV7 strains, 20 out of 1,014 aa (1.97%) were polymorphic between the strains. Comparison of the sequences in relation to the host revealed relevant differences between the strains infecting different common chimpanzee subspecies. Hence, 10 specific polymorphic sites (amino acid positions 128, 244, 538, 539, 692, 708, 803, 805, 859, and 1011) were constantly associated with PtroHV7 from *P. t. verus*, while two sites (at amino acid positions 596 and 861) were associated with the strain infecting *P. t. schweinfurthii* individuals (Table 4). Considering the viruses infecting *P. t. troglodytes* and *P. t. ellioti*, eight positions differentiated them from the PtroHV7 strains detected in *P. t. verus* and *P. t. schweinfurthii*. In addition, two different strains with specific polymorphic sites (six versus two) were identified from *P. t. troglodytes* and *P. t. ellioti* (Table 4). Nevertheless, both strains were detected in animals from the two last chimpanzee subspecies. Actually, chimpanzees Pte1 and Pte8, belonging to the *P. t. ellioti* subspecies, and Ptt4, of the *P. t. troglodytes* subspecies, were found to be infected with the same strain (GenBank accession numbers KJ843235, KJ843236, and KJ843234, respectively), while chimpanzees Pte3 (*P. t. ellioti*) and Ptt1 (*P. t. troglodytes*), from which we generated only a 1,298-bp fragment of the DNA polymerase gene, were found to be infected with the other strain (GenBank accession numbers KJ843233 and KJ843244, respectively) (see Table S2 in the supplemental material).

Phylogenetic analyses based on the glycoprotein B and DNA polymerase nucleotide sequences (not shown) provided a tree topology identical to that obtained from the nucleotide sequences (not shown) or the amino acid sequences of the full-length DNA polymerase only (Fig. 1). We therefore restricted analysis to the full-length DNA polymerase gene sequences from all the available roseoloviruses, using HHV6A and HHV6B as outgroup viruses. Phylogenetic analysis revealed that HHV7 and its African great ape homologues belong to a monophyletic lineage whose branching order corresponds to that of the primate host. Indeed, this analysis demonstrated the existence of three distinct lineages, supported by high posterior probability values, corresponding to the *Gorilla*, *Homo*, and *Pan* clades. In addition, the *Pan* lineage is subdivided into two branches leading to the *P. paniscus* and *P. troglodytes* viruses. The latter viruses group together on a monophyletic branch, which is divided into three distinct branches that correspond to the *P. t. verus*, *P. t. troglodytes*/*P. t. ellioti*, and *P. t. schweinfurthii* viruses. Furthermore, the analysis demonstrated that *Pan* viruses are the closest relatives of HHV7. All branching orders perfectly fit with the host species tree. Then, to further explore the cospeciation hypothesis, a time calibration analysis was performed. Gorilla viruses split from *Homo*/*Pan* viruses about 9.00 MYA (95% HPD, 5.96 to 12.03 MYA), while *Homo* separated from *Pan* about 6.98 MYA (95% HPD, 3.93 to 9.97 MYA), and *P. paniscus* viruses split from *P. troglodytes* viruses about 2.63 MYA (95% HPD, 1.27 to 4.32 MYA). The divergence between western and central/eastern chimpanzee viruses is estimated to be 1.02 million years (95% HPD, 0.44 to 1.79 MYA).

## DISCUSSION

Improvements to molecular diagnostic techniques have led to the discovery of a number of herpesviruses in NHPs in the last few decades. The present study, seeking to identify HHV7 homologues in African great apes, has characterized a novel roseolovirus close to HHV7 in each African great ape species and subspecies tested. The combination of degenerate PCR primers used for screening proved to be particularly useful in terms of both sensitivity (see Table S2 in the supplemental material) and specificity, with only HHV7-like sequences being identified, even though some of the tested animals were known to be infected with other herpesviruses (6, 22). The identification of distinct HV7 strains in African great apes, while expected, significantly expands our knowledge of the host range of this viral genus and has shed new light on herpesvirus evolution. Phylogenetic analyses of both glycoprotein B (not shown) and DNA polymerase sequences revealed that HHV7 and its African great ape homologues form distinct monophyletic lineages whose topological resemblance with the host phylogeny is highly suggestive of virus-host codivergence (Fig. 1). Our results substantiate and extend the findings of previous molecular studies on primate lymphocryptoviruses, cytomegaloviruses, and rhadinoviruses (6, 9, 16). To our knowledge, no study on primate herpesviruses published so far has extensively studied all species and subspecies of the *Pan* genus at the same time. This allowed us to decipher the relationships of the *Pan* viruses in detail at the host subspecies level. It is noteworthy that, despite the high level of sequence conservation between the different PtroHV7 strains, changes at specific amino acid positions allow one to unambiguously classify the different strains according to the host subspecies from which they were identified. In theory, this makes it possible to use the strain-specific polymorphism as a genetic signature to differentiate them (Table 4). These results were phylogenetically confirmed. Indeed, within the *P. troglodytes* clade, structuring of the PtroHV7 phylogeny by subspecies was also evident, with three distinct lineages corresponding to individual subspecies (*P. t. verus*, *P. t. schweinfurthii*, and *P. t. ellioti*/*P. t. troglodytes*) (Fig. 1). Unfortunately, we were not able to definitively associate PtroHV7 sequences from *P. t. ellioti* or *P. t. troglodytes* with the corresponding host subspecies. Actually, viral sequences from these two subspecies have been identified in individuals from both subspecies. Analysis of mitochondrial DNA (mtDNA) sequences from these animals, as previously described, confirmed their genetic origin, eliminating any misclassification (6). Therefore, these likely represent transmission events between these two chimp subspecies in both directions. Such events did not likely occur in nature, since the two subspecies are separated from each other by the Sanaga River in central Cameroon, which serves as a geographic barrier (42–45). However, this barrier is not complete, and exceptions have been reported (46). It is also possible that these animals were in contact during transportation or other

**TABLE 3** Nucleotide and amino acid identities between the different known primate roseoloviruses on the basis of the complete DNA polymerase gene sequence

| Virus | GenBank accession no. | % identity with: PtroHV7*v.* | | PtroHV7*e.* | | PtroHV7*t.* | | PtroHV7*s.* | | PpanHV7 | | GgorHV7 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Nucleotide | Amino acid | Nucleotide | Amino acid | Nucleotide | Amino acid | Nucleotide | Amino acid | Nucleotide | Amino acid | Nucleotide | Amino acid |
| PtroHV7*v.* | KJ843227, KJ843237 | 99.9 | 100 | 98.3 | 98.3–98.5 | 98.4 | 98.7 | 98.6 | 98.7 | 94.8–94.9 | 96 | 86.7–86.8 | 89.6 |
| PtroHV7*e.* | KJ843233, KJ843235, KJ843236 | 98.3 | 98.3–98.5 | 99.9–100 | 99.9–100 | 99.1 | 99–99.1 | 99.2 | 99–99.1 | 95.1–95.2 | 96.3–96.4 | 87.1–87.2 | 89.9–90 |
| PtroHV7*t.* | KJ843234, KJ843244 | 98.4 | 98.7 | 99.1 | 99–99.1 | 99.8[a] | 100[a] | 99.6 | 99.6 | 95.4–95.5 | 96.9 | 87.2–87.3 | 90.1 |
| PtroHV7*s.* | KJ843228, KJ843238, KJ843239, KJ843240 | 98.6 | 98.7 | 99.2 | 99–99.1 | 99.6 | 99.6 | 100 | 100 | 95.4–95.6 | 96.7 | 87.3–87.4 | 90.2 |
| PpanHV7 | KJ843229, KJ843230, KJ843241 | 94.8–94.9 | 96 | 95.1–95.2 | 96.3–96.4 | 95.4–95.5 | 96.9 | 95.4–95.6 | 96.7 | 99.9–100 | 100 | 86.5–86.6 | 89.4 |
| GgorHV7 | KJ843231, KJ843232, KJ843242, KJ843243 | 86.7–86.8 | 89.6 | 87.1–87.2 | 89.9–90 | 87.2–87.3 | 90.1 | 87.3–87.4 | 90.2 | 86.5–86.6 | 89.4 | 99.9–100 | 100 |
| HHV7 strain RK | AF037218 | 86.5 | 90.6 | 86.4–86.5 | 90.6–90.7 | 86.4 | 90.7 | 86.5 | 90.7 | 86–86.2 | 90.1 | 85.8–85.9 | 89.2 |
| HHV7 strain JI | U43400 | 86.4 | 90.5 | 86.3–86.4 | 90.5–90.6 | 86.4 | 90.6 | 86.5 | 90.6 | 86–86.2 | 90 | 85.7–85.8 | 89.1 |
| HHV6A strain U1102 | X83413 | 64.9 | 66.3 | 64.9–65 | 66.5–66.6 | 64.7 | 66.5 | 64.7 | 66.4 | 64.4 | 66.8 | 64.9–65 | 66.7 |
| HHV6B strain Z29 | AF157706 | 64.8 | 66.5 | 64.8–64.9 | 66.7–66.8 | 64.6 | 66.7 | 64.6 | 66.6 | 64.6 | 66.9 | 65–65.1 | 67 |
| HHV6-B strain HST | AB021506 | 64.8–64.9 | 66.5 | 64.9 | 66.7–66.8 | 64.7 | 66.7 | 64.7 | 66.6 | 64.7 | 66.9 | 65.1 | 67 |
| *Pan troglodytes* herpesvirus 6[b] | AY359407 | 66.1 | 70.6 | 66.2–66.3 | 70.6–70.9 | 66.3 | 70.9 | 66.3 | 70.9 | 65.5 | 70.6 | 65.8 | 71.3 |
| *Pan troglodytes* roseolovirus 1[c] | AY854171 | 65.8 | 66.8 | 65.7–65.8 | 66.8–67 | 65.7 | 66.8 | 65.8 | 66.8 | 65.5–65.6 | 67.2 | 65.6–65.7 | 67.2 |
| MndHVβ[d] | AF282942 | 58.8–59 | 62.3 | 58.4–58.6 | 60.3–61 | 59.4 | 62.3 | 58.8 | 62.2 | 59.2 | 62.3 | 60.5 | 62.3 |

[a] Numbers refer to values obtained by comparing the common 1,298-bp/432-aa sequences of PtroHV7 obtained from two *P. t. troglodytes* individuals.
[b] Comparison with the PanHV6 sequence was performed only on the basis of the 883-bp/294-aa common fragments.
[c] The comparison with these viral strains was performed on the basis of the common 2,214-bp/738-aa fragments.
[d] The comparison with these viral strains was performed only on the basis of the common 478-bp/160-aa common fragments.

**TABLE 4** Amino acid variability of the DNA polymerase between the different strains of PtroHV7 infecting the different *Pan troglodytes* subspecies

| *P. troglodytes* subspecies | Amino acid at the following position[a]: | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 16 | 128 | 244 | 349 | 538 | 539 | 596 | 606 | 622 | 679 | 692 | 708 | 802 | 803 | 805 | 859 | 861 | 927 | 963 | 1011 |
| *P. t. ellioti*, *P. t. troglodytes* | T/A | V | I | I/L | I | S | N | V/I | V/A | A/T | V | D | T/A | T | I | V | I | M/L | D/E | D |
| *P. t. schweinfurthii* | A | V | I | L | I | S | D | I | A | T | V | D | A | T | I | V | L | M | D | D |
| *P. t. verus* | A | I | L | L | L | N | N | I | A | T | I | A | A | R | V | G | I | M | D | A |

[a] Amino acid position numbers are relative to the methionine of the DNA polymerase.

circumstances. Many of the animals were confiscated from private owners, dealers, or hunters, and therefore, their former conditions of life are obscure. Another possibility is that these animals shared the same cage in the rescue center where they were brought and that these events correspond to direct transmissions between cage mates. Notably, species jumps between chimpanzees and gorillas have been proposed for CMVs (9). Therefore, to accurately associate the correct chimpanzee subspecies with its cognate virus, for a definitive confirmation of the variation repertoire, the characterization of additional PtroHV7 sequences from a larger series of wild Cameroonian common chimps of these two subspecies is essential.

Our data strongly support virus-host coevolution in terms of branching order but also in terms of timing evolution. Indeed, our Bayesian analysis dates the divergence between *Homo* and *Pan* viruses back to about 7 MYA. Our results, in spite of the large associated 95% HPD intervals, sit comfortably with those published by Langergraber et al. (47) on primate evolution derived from generation time estimates and mutation rates in wild chimps and gorillas as well as with those of Wilkinson et al. (40), based on an integrated analysis of paleontological and molecular data. Tellingly, these results overlap those obtained from similar analyses based on UL55 (glycoprotein B) or UL55 plus UL56 sequence alignments of primate CMVs (9). Of particular interest, our estimated timing of diversification of *P. paniscus* and *P. troglodytes* viruses of about 2.63 MYA (95% HPD, 1.27 to 4.32 MYA) is in agreement with, though it is slightly older than, host sequence divergence date estimates from previously published studies based on different types of data sets or models (43, 47–50). Finally, considering the viruses of *P. troglodytes*, their branching pattern is also similar to the host phylogeny, specifically in that the host tree has *P. t. verus* diverging first from the other lineages, i.e., from *P. t. ellioti*/*P. t. troglodytes* and then from *P. t. schweinfurthii*. Strikingly, the divergence time between common chimpanzee viruses is estimated to be about 1 million years (95% HPD, 0.44 to 1.79 MYA), a value indistinguishable from that inferred from analysis of complete mitochondrial genomes (43). Therefore, even if inconsistent results on the evolutionary history of common chimpanzees are reported due to differences in the types of data or assumption models applied and in spite of the limitations concerning the PtroHV7 strains of *P. t. troglodytes* and *P. t. ellioti* chimps mentioned above, our results strongly support the coevolution hypothesis of HHV7 and its African great ape homologues with their hosts (45, 50, 51). Unfortunately, we were not able to identify the sister clades of the *P. t. verus*/*P. t. ellioti* and *P. t. troglodytes*/*P. t. schweinfurthii* viruses, but as was already suggested, these limits can be alleviated by studying more individuals of the *P. t. ellioti* and *P. t. troglodytes* subspecies (43).

The present results strongly suggest a high prevalence rate of HHV7 homologues in their respective host species. Indeed, in spite of a certain sampling bias (the use of captive versus wild-caught animals and testing of only 10 animals for the *P. t. troglodytes* and *P. t. ellioti* subspecies) and of a wide range of rates (20% for the lowest prevalence rate versus 78% for the highest prevalence rate), the average molecular prevalence of HHV7 homologues estimated here is 45.9%. This result, though substantially lower than that for HHV7, is concordant with what is reported for HHV7. Indeed, although the seroprevalence of this viral infection in the adult human population is assumed to exceed 90%, the rate of HHV7 PCR positivity is lower, ranging from 50 to 70% (52, 53). Serum and plasma samples were not available from the animals evaluated in this study. Nevertheless, determination of the prevalence rates of these different viruses in their hosts by a serological approach fully deserves further investigations. It could therefore be valuable, despite the absence of specific serological tools, to test African great ape sera for cross-reactivity against HHV7 antigens, as has already been done for other herpesviruses (6, 7, 21).

From a taxonomic viewpoint, our results are of major importance. In view of the increasing number of herpesviruses, the *Herpesvirales* study group of the International Committee on Taxonomy of Viruses (ICTV) has discussed ways of updating the herpesvirus species definition and the methods used for taxonomic assignment (1, 54). In addition, the ICTV adopted a new naming system in which NHP herpesvirus species are named after the host genus, with the name ending in "-ine." Therefore, if we consider only the chimpanzee and gorilla herpesviruses that are currently recognized as species by the ICTV, i.e., Panine herpesviruses 1 and 2 and Gorilline herpesvirus 1, the novel Panine viruses described in this paper would have the minimally informative names of Panine HV3 through HV7, and the gorilla virus would have the name Gorilline herpesvirus 2. This naming system is not perfectly intelligible with respect to the host and virus parts of the equation. In addition, we have to disregard the different cytomegaloviruses, lymphocryptoviruses, and rhadinoviruses that have been detected only by PCR and for which there is not sufficient sequence information for them to be recognized as viral species, as well as the *Pan troglodytes* herpesvirus 6/*Pan troglodytes* roseolovirus 1 (unclassified *Roseolovirus*) and the chimpanzee α1 herpesvirus, which has an unclassified *Alphaherpesvirinae* status. From these observations, for the readability of the paper, we felt that it made good sense to employ host species-based names. It actually seems relevant to have informative names that have practical value until formal names can be adopted. In this informal naming system, the viruses were named after the host species followed by two uppercase letters corresponding to the viral family (HV for herpesvirus), to which they were then assigned the Arabic numeral 7 to differentiate viruses from different host species and to identify viruses belonging to the same viral genus (Table 1). Finally, to differentiate viruses infecting *P. troglodytes*, we have tentatively associated the host subspecies information with the name of the virus. The first letter of the subspecies (e.g., *v.* for

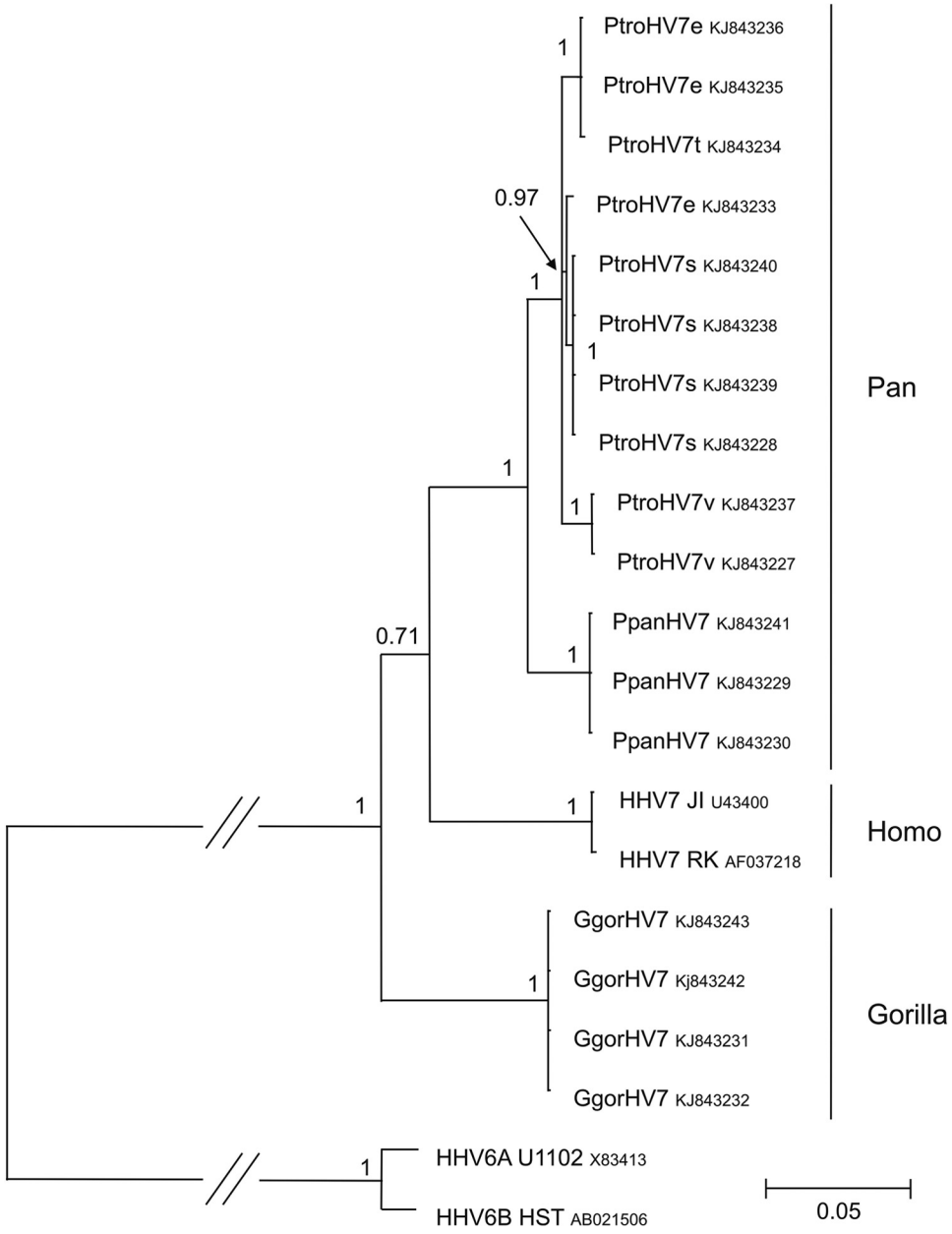


**FIG 1** Phylogenetic tree constructed using Bayesian methods (10,000,000 replicates; Mr. Bayes software, v3.2.2) and on the basis of 1,014 aa of the DNA polymerase of all published sequences of roseoloviruses. The tree is based on the JTT model with a G distribution of amino acid evolution. The virus names are associated with their accession numbers. Support for the nodes is provided by the posterior probabilities of the corresponding clades. All resolved nodes have posterior probabilities of greater than 0.7. The scale bar indicates the amino acid sequence divergence among sequences.

*verus*) in lowercase has been added. For example, PtroHV7 from *P. t. verus* is written PtroHV7*v*. Considering viral strains from *P. t. troglodytes* and *P. t. ellioti*, this system has also been applied even if the cross identification of the two viral variants in both subspecies is a major limitation. These data thus open the discussion on the usefulness and readability of the current ICTV nomenclature for primate herpesviruses.

In conclusion, this study provides a definitive identification of HHV7-related viruses in nonhuman primates, specifically, in African great apes. We conclusively demonstrate the cospeciation between these new viruses and their hosts and report cases of cross-species transmission between two common chimpanzee subspecies in both directions. These viruses are, in addition, highly prevalent in their respective host species. In light of these data, we can hypothesize that each simian species probably harbors its own variant of HV7 that remains to be discovered.

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A.L. and V.L. conceived of and designed the experiments. D.D. and V.L. performed the experiments. D.D., A.L., and V.L. analyzed the data.

A.G., H.N., E.N., and E.J.V. contributed biological materials. A.L., A.G., E.J.V., and V.L. wrote the paper, and all authors participated in its final writing and editing.

We have no competing interests to declare.

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